

FIRST REPORT OF CONNEXIN 43-POSITIVE GASTROINTESTINAL STROMAL TUMOR (GIST)

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SUMMARY – Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms most frequently found in the stomach and presenting without symptoms or with unspecific ones such as hemorrhage and abdominal pain. The malignant potential of GIST is variable and there are several prognostic indexes for treatment and follow up. The superior diagnostic method is endoscopic ultrasound combined with fine needle aspiration biopsy (EUS-FNA) immunocytochemistry. Surgery is the preferable treatment option, while recurrent or advanced disease is best managed with tyrosine kinase inhibitors. We report a case of a 20-year-old man that presented with gastrointestinal bleeding and anemia. Upper gastrointestinal endoscopy detected a submucosal lesion and computerized tomography revealed a node near the liver. EUS-FNA immunocytochemistry found CD-117 positive cells suggestive of GIST and the patient was operated on. The diagnosis was confirmed by histopathology with immunostaining. Prognostic assessment was done according to tumor size and Ki-67 index with mitosis count on microscopy. Many studies have shown that tumors demonstrate an abnormal number, structure and occurrence of connexin proteins with altered connexin-mediated intercellular communication. Since a great deal of gastroenterological tumors express connexin 43, our aim was to find out whether it was present in GIST. Immunohistochemically, the tumor was positive for connexin 43, which was not previously described as typical. The early postoperative course was uneventful, free from complications. At three-year postoperative follow-up, the patient was subjectively well and without clinical signs of disease recurrence.

Key words: *Gastrointestinal stromal tumors – diagnosis; Gastrointestinal stromal tumors – pathology; Neoplasms; Connexin 43; Immunohistochemistry*

Introduction

Subepithelial lesions of the gastrointestinal tract are commonly due to mesenchymal overgrowth of either neurogenic, muscular tissue or gastrointestinal stromal tumors. The incidence of gastrointestinal stromal tumors (GISTs) is around 15-20 cases *per* million population, most frequently found in gastric submucosa. Although diagnostically resembling leiomyoma, leiomyosarcoma or neurogenic tumors, it has

different immunohistochemical profile. The c-kit proto-oncogene product CD-117 is a particular marker of GIST with predilection to the interstitial cell of Cajal as intestinal motility pacemaker cell. Besides its biological rarity, there is still dispute regarding the diagnosis, malignancy assessment, clinical behavior and treatment options¹.

Case Report

A 20-year-old man presented with melena and anemia (Hb 80 g/L, Hct 0.226%, MCV 77 fl). Sub-

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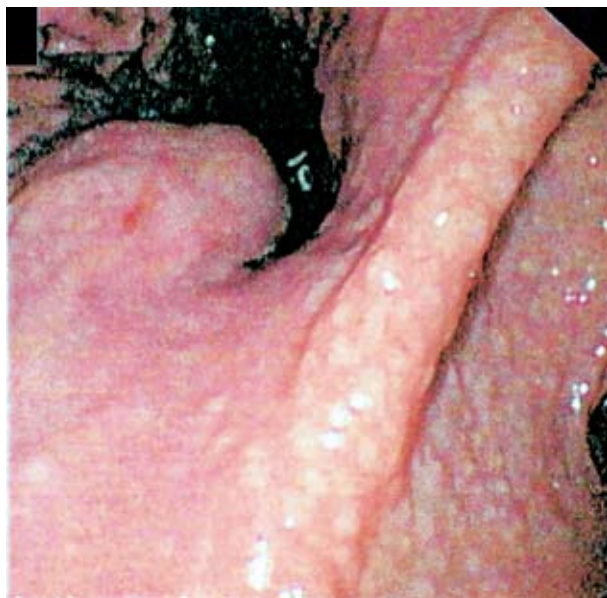


Fig. 1. Upper gastrointestinal endoscopy showing resistant submucosal protuberance on the anterior part of the gastric body.

jectively, major symptoms were unspecific, having persisted for 3–4 weeks prior to admission. Tar colored stools occurred dozen of times and resolved spontaneously. Gastroscopy revealed increased mucosal resistance with mild ulceration covered with fibrin in the anterior part of the gastric body (Fig. 1). Histo-

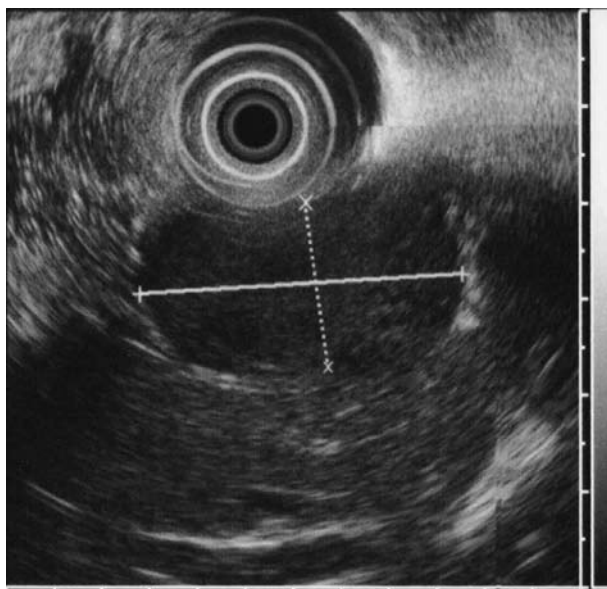


Fig. 2. Endoscopy ultrasound of submucosal hypoechoic mass in the 4th EUS layer.

logic biopsy was non-diagnostic, pointing to chronic erosive gastritis with mononuclear infiltration. Abdominal ultrasound and MSCT found a solid nodule of 37x24 mm in diameter below the lower left liver lobe. Endoscopic ultrasound (EUS) revealed a hypoechoic solid submucosal lesion with regular borders and homogeneous echotexture in the 4th layer (Fig. 2). EUS fine needle biopsy (FNAB) revealed spindle cells with c-kit; CD 117 positive immunostaining (Fig. 3A, B). There were no signs of local or distant tumor spread.

The patient was referred for surgery. Laparoscopic stapler resection was performed, with clear cut margins, leaving the tumor pseudocapsule intact. The operative procedure lasted 60 min, there was no need for blood transfusion and the patient was released from the hospital on postoperative day 3. Macroscopically, the tumor was a pallid single node. Histologically, oval-shaped cells predominated, with a relatively small percentage of spindle cells in muscularis propria and subserous layers. There were scarce microscopic hemorrhages without necroses, 5 mitoses *per* HPF and 5% activity in Ki-67 cellular index. Immunohistochemistry antigen staining was positive for c-kit; CD 117, CD 34, and negative for SMA and S100. Histopathologic evaluation indicated a low-risk aggressive GIST.

The patient has been followed-up on outpatient basis for three years now. Routine clinical examination showed no evidence for disease recurrence, and anemia resolved with oral iron supplementation. Gastroscopy and abdominal ultrasound found optimal local postoperative result.

Discussion

Proliferative protuberances in the gastrointestinal tract were once classified as mesenchymal tumors. Although similar on common hemalaun-eosin staining, the introduction of immunohistochemistry has offered markers of cellular determination, e.g., neural (S-100), muscular (SMA), and the most common among them positive CD-117 antigen termed gastrointestinal stromal tumor (GIST), ontogenetically related to interstitial pacemaker cells of Cajal.

As in our case, gastrointestinal endoscopy usually offers little to no diagnostic information, since biopsy samples are obtained from layers above the lesion. In comparison with abdominal ultrasound, MSCT or

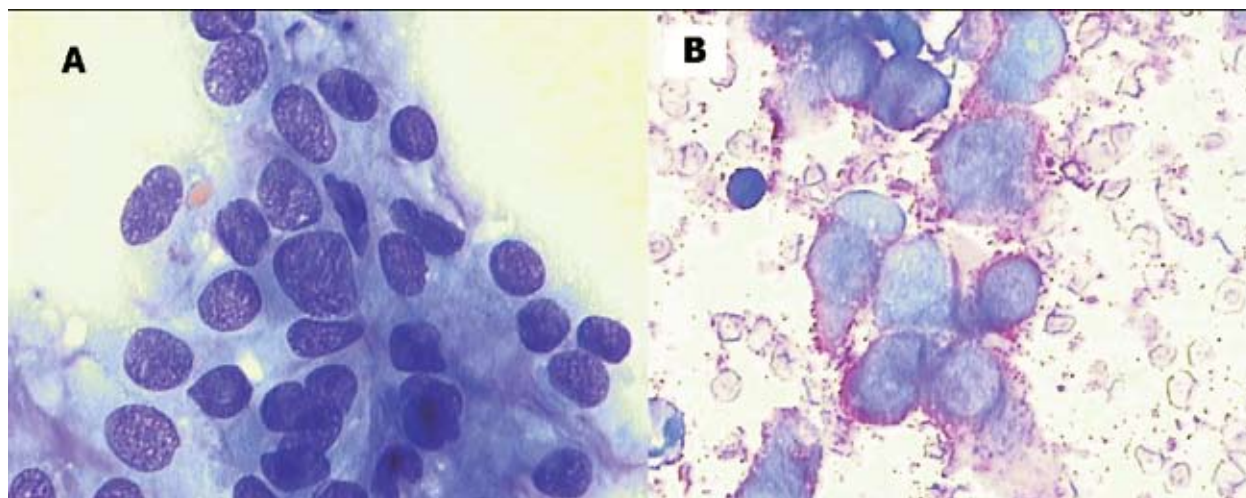


Fig. 3. Cytologic features: (A) May-Grünwald-Giemsa (MGG) stain (magnification X400); (B) immunocytochemistry was positive for CD 117 (magnification X1000).

MRI, EUS is the most sensitive imaging tool to differentiate submucosal lesions, excluding extramural compression and suggesting non-tumorous lesions². Certain endoscopic ultrasound morphological characteristics such as tumor size >3 cm, irregular extraluminal border and cystic spaces are suggestive of a higher malignant potential³. GISTs are located at either 2nd or 4th EUS layer (muscularis mucosa, muscularis propria).

The patient presented had a GIST greater than 30 mm, located in the 4th EUS layer, with ulcerated gastric mucosa, thus not being eligible for endoscopic mucosal resection (EMR), so he was referred to surgery department. Minimally invasive laparoscopic surgery is the first-line treatment option in managing GISTs of ≥ 2 cm in diameter and originating from the 2nd EUS layer, i.e. unresectable by EMR.

The malignant potential of GIST is diverse and most authors agree that it depends on tumor size, mitotic activity, and presence of metastases. According to the commonly agreed international prognostic groups, our patient had a low risk of recurrence and aggressive behavior⁴; yet, due to his young age and unusual, i.e. malignant (EUS-FNA) cause of bleeding, we did not insist on definitive preoperative prognostic histopathology score. Accurate histologic diagnosis is not considered to be mandatory parameter for preoperative procedure. One must not disregard the fact that too small preoperative samples may also be inconclusive. Postoperative histology found a low Ki-67

cellular index score (<5%), which also confirmed the low risk prognostic assessment. Since Ki-67 index was low, it was paired with the histologic mitotic count to rule out false negative result⁵.

Connexin proteins are involved in the regulation of cell homeostasis, proliferation, differentiation, apoptosis, diffusion of molecules and cytokines⁶. Many studies have shown that tumors of various organs or tumor cell lines exhibit an abnormal number, struc-

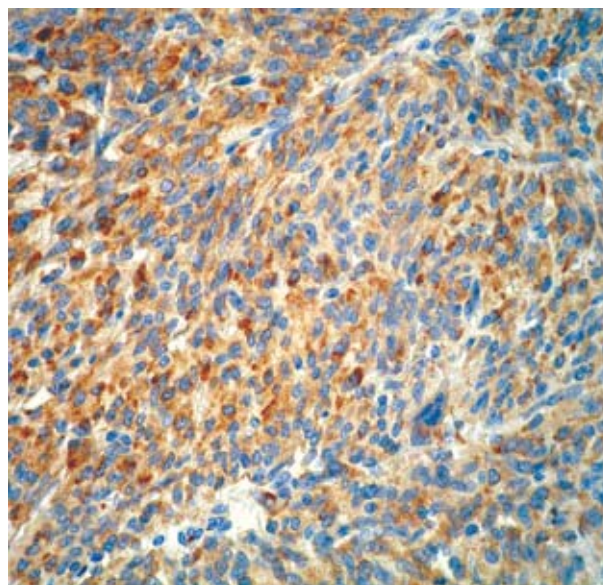


Fig. 4. Histopathology, light microscopy (magnification X400); immunohistochemically positive intracytoplasmic reaction for connexin Cx43 in all cells.

ture and occurrence of connexin proteins with altered connexin-mediated intercellular communication⁷.

Connexin 43 (Cx43) is considered one of the most widely expressed gap junction proteins, with different and as yet incompletely understood responsibilities in the regulation of cell growth, apoptosis and malignant growth suppression⁸. Furthermore, as a great deal of gastroenterological tumors express Cx43, our aim was to find out if it was present in gastrointestinal stromal tumor of the stomach. In our case, we performed immunohistochemistry analyses for connexin proteins Cx43 and Cx59. Positive intracytoplasmic reaction to Cx43 was recorded in all visible cells (Fig. 4), which was not previously described as typical for stomach GIST⁹. Although firm conclusions could not be made without greater population series, Cx43 seems to be expressed in stomach GIST of low grade. Our case of GIST did not show immunohistochemical reaction to Cx59.

Sentinel follow up of particular interest should be focused on the first two postoperative years, since the majority of recurrences occur in this period¹⁰. If tumor margins on surgical resection are intact, the 5-year survival was estimated to be 54% in a sample of 200 cases¹¹.

Chemotherapy is reserved for disseminated metastatic disease or recurrent postoperative GIST^{12,13}. Tyrosine kinase (KIT, PDGFR α , PDGFR β) inhibitors such as imatinib-mesylate are the treatment of choice at the moment. Treatment success can be seen as early as one month of therapy initiation by contrast enhanced CT scan.

In conclusion, positive Cx43 immunohistochemistry in stomach GIST might serve as a potential indicator regarding the diagnosis, therapy or prognosis, but further analyses are needed to clarify its role. Additional studies are warranted to elucidate the significance of connexin in GIST malignant behavior or treatment course.

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Sažetak

PRVI PRIKAZ SLUČAJA PROBAVNOG STROMALNOG TUMORA (GIST) POZITIVNOG
NA KONEKSIN 43

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Gastrointestinalni stromalni tumor (GIST) spada u novotvorine mezenhimnog podrijetla, a najčešće se nalazi u želucu. Često bude bez simptoma ili se prezentira paletom nespecifičnih simptoma poput gastrointestinalnog krvarenja ili boli u abdomenu. Maligni potencijal GIST-a je raznolik te postoji nekoliko prognostičkih indeksa za dijagnostiku i praćenje. Najznačajniju dijagnostičku metodu čini endoskopski ultrazvuk kombiniran s citološkom biopsijom. S terapijske strane najpoželjniji izbor je kirurško liječenje, a za uznapredovale ili recidivne oblike bolesti liječenje je farmakološko inhibitorima tirozin-kinaze. Ovdje se prikazuje slučaj 20-godišnjeg mladića koji je došao na obradu zbog gastrointestinalnog krvarenja i anemije. Gastroskopijom se otkrila submukozna promjena koja se na kompjutoriziranoj tomografiji prikazala kao čvor uza stražnji rub jetre. Citopunkcijom uz pomoć endoskopskog ultrazvuka nađene su stanice pozitivne na CD-117 koje ukazuju na GIST, te je bolesnik operiran. Dijagnoza je potvrđena patohistološki uz imunohistološko bojanje. Prognostička patohistološka procjena je učinjena prema veličini tumora, Ki 67 mitotskom indeksu. Brojne studije su pokazale kvantitativne ili kvalitativne promjene koneksinskih proteina te promijenjene međustanične komunikacije posredovane tijesnim vezama. Kako je u velikom broju tumora probavne cijevi primijećena promjena ekspresije koneksina Cx43, naš je cilj bio ispitati zastupljenost u GIST-u. Naš slučaj bio je pozitivan na Cx43, što nije ranije opisano kao očekivano. Rani poslijeoperacijski tijek u bolesnika je bio bez komplikacija, a dosad se kroz ambulantno praćenje unatrag 3 godine osjeća dobro, kontrolni nalazi laboratorija su uredni, te se kliničkom obradom ne nalazi znakova recidiva bolesti.

Ključne riječi: *Gastrointestinalni stromalni tumori – dijagnostika; Gastrointestinalni stromalni tumori – patologija; Novotvorine; Koneksin 43; Imunohistokemija*

